The hen’s egg chorioallantoic membrane (HET-CAM) test to predict the ophthalmic irritation potential of cysteamine-containing gels and solutions: Quantification using Photoshop® and ImageJ.

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ABSTRACT SUMMARY

A modified hen’s egg chorioallantoic membrane (HET-CAM) test has been developed, combining ImageJ analysis with Adobe® Photoshop®. The irritation potential of an ophthalmic medicine can be quantified using this method, by monitoring damage to blood vessels. The evaluation of cysteamine containing hyaluronate gel is reported. The results demonstrated that the novel gel formulation is non-irritant to the ocular tissues, in line with saline solution (negative control). In conclusion, the modification of the established HET-CAM test can quantify the damage to minute blood vessels. These results offer the possibility to formulate cysteamine in an ocular applicable gel formulation.

INTRODUCTION

An eye gel has been developed, and is in the process of characterisation in our laboratories (1). Part of this process is to elucidate the ophthalmic irritation potential of the eye gel. The HET-CAM test, an inexpensive and sensitive assay which uses fertilised hen’s eggs is an established test for ophthalmic irritancy, and has shown good correlation to ophthalmic irritation in the vivo situation (2). The membrane separates the embryo from the internal airspace, and is non-innervated, highly vascularised and responds to injury in a similar way to rabbit conjunctiva (3).

EXPERIMENTAL METHODS

Various medicated and non-medicated solutions and gels were manufactured, at a range of different pH values. Fertilised hen’s eggs were incubated at 37±0.5°C and 40%±5% humidity for 9 days (4). On day 10, the shells were cut just above the marked line of the chorioallantoic membrane (Figure 1). After the addition of 2ml of 0.9% saline solution, the same volume of gel was then added directly onto the CAM, and a timer started. Any lysis, haemorrhaging and/or coagulation at different times over a 5-minute period after application of the test solution will be documented.

RESULTS AND DISCUSSION

A semi-qualitative analysis was performed using the photographs, where the severity of any haemorrhage was graded on a scale from 0 (no reaction) to 3 (strong reaction) using the method developed by Gupta et al (5). The photographs were subsequently analysed using Adobe® Photoshop® and ImageJ to quantify the exact level of vascular damage, using a forensic science technique to allow a more detailed and robust analysis of the gels to be made (6).

Figure 1. Image showing the exposed chorioallantoic membrane.
The medicated solution at pH 6.45 caused no hemorrhaging, however it is interesting to note that there was a marked swelling of the yolk and vitelline membrane. This ‘turgidity’ effect was caused by the hypotonic nature of the solution. This solution was made as per the formulation of the commercial eye drop solution, the pH of which was found to be higher than the published value of 4.5 at 6.45 (7). The irritation experienced by patients could be due more to the hypotonic nature of the solution, and subsequent swelling of the ophthalmic tissues, rather than the low pH of the drops. As a comparison, the same ophthalmic solution was tested with a pH of 4.75. The medicated solution at pH 4.75 caused minor haemorrhaging and turgidity of the yolk and vitelline membrane, as well as marked hyperaemia. The current ophthalmic solution has a pH beyond the limits of ocular tolerance of 6.6-7.8 (8), which produces minor eye irritation. The HET-CAM tests demonstrate that the ophthalmic solution is also hypotonic, and causes discomfort from tissue swelling.

The study demonstrates that the new formulation is non-irritant and well tolerated.

CONCLUSION

A new ophthalmic gel formulation has been developed to treat the corneal crystals associated with cystinosis. As part of ongoing tests within our laboratories, the ocular irritation potential of the gel was determined using a modified HET-CAM test. The new formulation outlined in this paper offers the possibility of a reduced daily dosage schedule (once or twice a day) and reduced ocular toxicity (zero score, non-irritant), which may improve patient compliance. This in turn may improve long-term morbidity. The modified HET-CAM test the can quantify the damage to minute blood vessels, and demonstrated that the new formulation is safe for in vivo use.

REFERENCES


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